

UCSF

UC San Francisco Previously Published Works

Title

Formation of slow-reacting substance of anaphylaxis in human lung tissue and cells before release.

Permalink

<https://escholarship.org/uc/item/06t6z68s>

Journal

The Journal of experimental medicine, 140(5)

ISSN

0022-1007

Authors

Lewis, RA
Wasserman, SI
Goetzi, EJ
et al.

Publication Date

1974-11-01

DOI

10.1084/jem.140.5.1133

Peer reviewed

FORMATION OF SLOW-REACTING SUBSTANCE OF ANAPHYLAXIS IN HUMAN LUNG TISSUE AND CELLS BEFORE RELEASE*

By ROBERT A. LEWIS,† STEPHEN I. WASSERMAN,‡ EDWARD J. GOETZL,§ AND K. FRANK AUSTEN

(From the Departments of Medicine, Harvard Medical School and Robert B. Brigham Hospital, Boston, Massachusetts 02120)

Slow-reacting substance of anaphylaxis (SRS-A)¹ is an acidic, sulfur-containing chemical mediator with an approximate mol wt of 500-600 (1, 2), which is released during immediate hypersensitivity reactions and has the capacity to increase vascular permeability (3) and to contract certain smooth muscle preparations (4). The release of SRS-A in vitro was initially detected by analysis of the perfusates obtained from sensitized guinea pig heart-lung preparations (5, 6) and the diffusates of isolated segments of guinea pig lung (7) challenged with specific antigen. Subsequently, SRS-A was released in vivo into the peritoneal cavity of the hyperimmunized rat by intraperitoneal challenge (8) and into the plasma of the sensitized guinea pig undergoing systemic anaphylaxis in response to intravenous antigen (9). Studies with passively sensitized guinea pig (10) and primate lung fragments, monkey (11) or human (12, 13), demonstrated that IgG1 and IgE, respectively, prepared the tissues for subsequent antigen-induced release of SRS-A. More recently, material with the functional activity of SRS-A has been released by antigen challenge of sensitized monkey lung suspensions containing mast cells (14) and human leukocyte preparations rich in sensitized basophils (15, 16). SRS-A has not been detected in tissues (7) or cells, normal or sensitized, as a preformed chemical mediator; and its release in vitro occurs more slowly than that of the preformed chemical mediators, histamine and eosinophil chemotactic factor of anaphylaxis (ECF-A) (7, 17), consistent with a requirement for formation before release.

Although knowledge of the physicochemical characteristics of SRS-A and the immunoglobulins and critical cell types responsible for its release has evolved, there has been no demonstration of its cellular generation before release. Alteration of the tissue extraction procedure for residual mediators by the

*Supported by grants AI-07722 and AI-10356 from the National Institutes of Health.

†Postdoctoral trainees supported by Training Grant AI-00366 from the National Institutes of Health.

§Investigator, Howard Hughes Medical Institute.

¹Abbreviations used in this paper: DFP, diisopropyl fluorophosphate; ECF-A, eosinophil chemotactic factor of anaphylaxis; SAG, atopic serum from a ragweed-sensitive patient; SRS-A, slow-reacting substance of anaphylaxis.

institution of freeze-thawing six times to eliminate boiling or acid treatment revealed the accumulation of SRS-A after an IgE-dependent reaction in lung fragments or isolated lung cells. Thus, it was possible for the first time to define the time-course of the intracellular formation of SRS-A which precedes its release.

Materials and Methods

Chymopapain, collagenase, and catalase (Worthington Biochemical Corp., Freehold, N. J.), elastase (Sigma Chemical Co., St. Louis, Mo.), pronase, deoxyribonuclease, and N⁶,O²-dibutyryl cyclic 3',5'-adenosine monophosphate (dibutyryl cyclic AMP, Calbiochem, San Diego, Calif.), gelatin (Difco Laboratories, Detroit, Mich.), sodium metrizoate (Triosil-75, Glaxo Laboratories, Ltd., Greenford, England), sodium diatrizoate (Hypaque, Winthrop Laboratories, New York), Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), silicic acid (100-200 mesh) and Amberlite XAD-2 (Mallinckrodt Chemical Works, St. Louis, Mo.), histamine acid phosphate (Mann Research Labs, Inc., New York), atropine sulfate (Sigma Chemical Co.), mepyramine maleate (Merck, Sharp & Dohme, West Point, Pa.), no. 80 brass sieve (Matheson Scientific, Inc., Stoneham, Mass.), 8 μ m pore-size micropore filters (Millipore Corp., Bedford, Mass.), and five times recrystallized ovalbumin (Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill.) were purchased from the manufacturers. Ficoll-Hypaque cushions were made as described by Böyum (18). Ragweed antigen E (Research Resources Branch, NIAID, Bethesda, Md.), and 8-bromo-cyclic 3',5'-guanosine monophosphate (8-bromo-cyclic GMP, L. Simon, ICN Nucleic Acid Research Institute, ICN Corp., Irvine, Calif.) were provided as noted. All solvents used in silicic acid chromatography of SRS-A were of nanograde quality. The atopic serum from a ragweed-sensitive patient (SAG) had a concentration of 250 ng IgE protein/ml by radioimmunodiffusion (19). Rabbit antihuman IgE, provided by Dr. Peter H. Schur, Robert B. Brigham Hospital, Boston, Mass., was made against purified Shackford IgE myeloma (20), precipitated in 40% ammonium sulfate, and then subjected to immunoabsorption with normal human serum coupled to Sepharose 6B (21). The absorbed rabbit anti-IgE gave no precipitin lines on immuno-electrophoretic analysis with normal human serum. Tyrode's buffer (22) was used throughout unless otherwise noted.

Mediator Bioassays. The isolated atropinized guinea pig ileum was used to bioassay histamine, and the same preparation with the addition of an antihistamine was employed to quantitate SRS-A (10). Prostaglandin activity was assayed on the isolated ascending colon of the gerbil (23). ECF-A was assessed by a modification (24) of the Boyden chamber chemotactic technique (25) with purified human eosinophils (26).

SRS-A in diffusates or extracts of tissue or cells was characterized further by purification (1) of activity extracted into 80% ethanol. The extract was centrifuged at 19,000 *g* for 10 min at 4°C; and the supernate was flask evaporated, resuspended in distilled water, exposed to 0.1 N NaOH at 37°C for 30 min, and applied to a 10 ml Amberlite XAD-2 column. After a water wash of 30 ml, the activity was eluted with 30 ml of 80% ethanol, flask evaporated, resuspended in 1.1 ml of 100% ethanol, and applied to a 10 ml silicic acid column. 30 ml of each of the following solvents were then applied in a stepwise fashion: hexane, methylene chloride, acetone, n-propanol, and ethanol:concentrated ammonia:water (in a 6:3:1 vol/vol ratio). Each 30-ml eluate was flask evaporated and suspended in Tyrode's buffer for bioassay.

IgE-Dependent Reactions in Human Lung Fragments and Cell Preparations. Specimens of grossly normal-appearing human lung were obtained from patients at the time of surgery for lung cancer. The lung tissue was washed and fragmented as previously described (13). Reversed anaphylactic mediator release was carried out with duplicate 250- or 500-mg portions of replicate fragments incubated with a final dilution of 1:50 rabbit antihuman IgE in a vol of 1 ml at 37°C for the time period stated. For direct anaphylactic mediator release from passively sensitized lung fragments, 250- or 500-mg replicates were sensitized in 1 ml of SAG serum diluted 1:3 for 18 h at room temperature, washed three times, and challenged at 37°C with 200 ng ragweed antigen E in a final vol of 1 ml for the time stated. To terminate either direct or reversed release, the reaction mixtures were brought to 3 ml with iced Tyrode's buffer, and the supernates were decanted from the fragments. The

tissues were resuspended in 3 ml of Tyrode's buffer and freeze-thawed six times in a dry ice-acetone bath to disrupt the cells and recover the mediators for bioassay.

The method of Gould et al. (27) to isolate rabbit lung cells was applied with minor modifications to human lung. Washed human lung fragments suspended in Tyrode's buffer were exposed to 2.0 mg/ml pronase and 0.5 mg/ml chymopapain for 20 min at 37°C with stirring. Freed cells were separated from residual fragments by sieving through a no. 80 brass fine wire-mesh sieve and were washed three times in Tyrode's buffer. The residual fragments were treated with 1.0 mg/ml collagenase, 10.0 U/ml elastase, 0.03 mg/ml deoxyribonuclease, and 0.1 mg/ml catalase for 20 min at 37°C with stirring. Freed cells were again separated from the residual fragments by sieving and washing, and the cells from both digestion steps were combined and diluted to 10^7 nucleated cells/ml in Tyrode's solution made in 0.1 g/100 ml in gelatin (Tyrode's gelatin). 2.5 ml of cell suspension were layered over 2.5 ml of Ficoll-Hypaque and centrifuged at 400 *g* for 30 min at room temperature. Cells at the Tyrode's-Ficoll-Hypaque interface were aspirated with an 18-gauge needle, while cells in the pellet at the bottom of the tube were obtained by resuspension after the fluid layers had been discarded. Cells from the interface were identified with Wright's stain or toluidine blue as mast cells, macrophages, lymphocytes, lung parenchymal cells, and lung stromal cells, with only minor erythrocyte contamination; these cells contained more than 70% of the histamine and ECF-A in the starting mixture. Cells sedimenting to the bottom were predominantly erythrocytes and polymorphonuclear leukocytes and contained less than 5% of the original histamine and ECF-A. Cells from the interface were resuspended in Tyrode's gelatin and, after three washes to remove the Ficoll-Hypaque, were used for mediator release. In passive sensitization experiments, 25×10^6 cells were incubated in duplicate in a 1-ml vol of SAG serum diluted 1:3 at 37°C for 90 min, washed three times with Tyrode's gelatin, and then challenged with 200 ng of ragweed antigen E at 37°C for 30 min in a final vol of 1 ml Tyrode's gelatin unless otherwise noted. In reversed anaphylactic mediator release, 25×10^6 cells were incubated in duplicate in 1 ml of rabbit antihuman IgE in a final dilution of 1:50 for the time indicated. In order to terminate the release reactions, the suspensions were diluted to 3 ml by the addition of iced Tyrode's gelatin, and the cells were sedimented at 400 *g* for 5 min at room temperature. The supernates were decanted and the cell pellets resuspended in 3 ml of Tyrode's gelatin and freeze-thawed in a dry ice-acetone bath six times. Both supernates and tissue or cell extracts were assayed for the mediators of immediate hypersensitivity. The quantities of SRS-A in supernates and cell extracts were expressed as units obtained from the cells freed from 10 g of lung tissue (U/10 g lung equivalent). The number of lung cells freed and recovered after Ficoll-Hypaque purification varied from 15 to 50×10^6 cells/10 g of lung tissue with 4-8% being mast cells.

Results

SRS-A Generation and Release. The knowledge that SRS-A was not a preformed mediator but yet appeared in the diffusate within minutes after an immediate-type hypersensitivity reaction in tissue implied either a brief period of intracellular residence, extracellular formation, or a failure of existing methodologies to extract SRS-A in its tissue phase. The previously employed techniques of boiling for 10 min in Tyrode's buffer with or without acidification (7) as well as a freeze-thawing procedure adopted for the extraction of ECF-A (28) were used to seek SRS-A in normal and sensitized lung tissue before and after challenge. Sensitized tissues exposed to specific antigen not only released SRS-A into the supernate but disclosed tissue SRS-A with extraction by the freeze-thawing technique. Accordingly, it was possible to examine the cellular generation of SRS-A before its release from human lung tissue or isolated cells by direct and reversed anaphylactic reactions.

ACTIVATION BY ANTI-IGE. The time-course of total SRS-A generation in human lung fragments, as determined by the sum of the SRS-A remaining in the tissues and that released into the supernate after challenge of the tissues with anti-IgE is

shown in Fig. 1. SRS-A was undetectable in the tissues at time zero and accumulated rapidly, reaching the maximum tissue level observed within 2 min after the addition of anti-IgE. In contrast, SRS-A release into the supernate was not evident until 2-5 min and proceeded to increase until 10 min, reflecting continued generation. Histamine release occurred within the 1st min and reached near maximum at 2 min, paralleling cellular SRS-A generation before release.

Isolated cells challenged with the 1:50 dilution of anti-IgE (Fig. 2) demonstrated an accumulation of cellular SRS-A at 1 min with a plateau at 2 min. Release of SRS-A began concomitant with cellular accumulation and proceeded to rise throughout the 30 min of observation, implying continued generation. ECF-A and histamine release (not shown) were maximal at 2 min, reaching, respectively, 34% and 18% net total release, and plateauing with cellular SRS-A at a time when SRS-A generation was continuing. In two additional experiments,

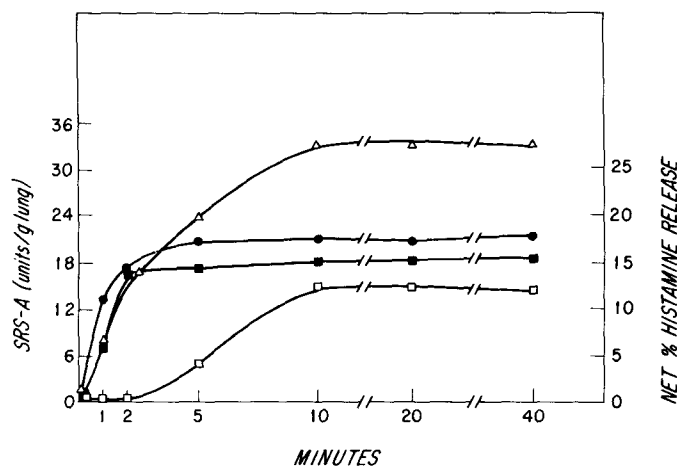


FIG. 1. Time-course of generation and release of SRS-A from human lung fragments challenged with anti-IgE. ■, SRS-A residual; □, SRS-A release; Δ, SRS-A total; and ●, histamine release.

cellular SRS-A again plateaued at approximately 2 min, with generation continuing as assessed by increasing release for 15-30 min.

The failure to demonstrate the accumulation of SRS-A in cells before release by a 1:50 dilution of anti-IgE in kinetic experiments prompted an alternative approach in which the intensity of the reversed anaphylactic activation was varied. Isolated cells challenged with a 1:1,000 or 1:2,000 dilution of anti-IgE accumulated SRS-A without release (Fig. 3). As the antibody concentration was increased to 1:100 or 1:200, there was an approximately equal distribution of SRS-A between cells and supernate, while with a 1:20 dilution of anti-IgE, all of the SRS-A detected was in the supernate. As the total SRS-A generated, the sum of that in the cells and supernate, was essentially the same with antibody concentrations from 1:200 to 1:20, and approximately three times that in the cells activated with antibody concentrations of 1:1,000 or 1:2,000, it is evident that the intensity of activation influences both the total quantity of SRS-A generated and the percentage released at 30 min.

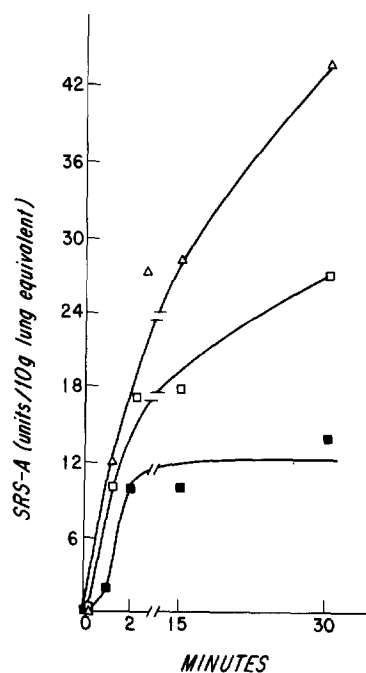


FIG. 2. Time-course of generation and release of SRS-A from isolated human lung cells challenged with anti-IgE. ■, SRS-A residual; □, SRS-A release; and Δ, SRS-A total.

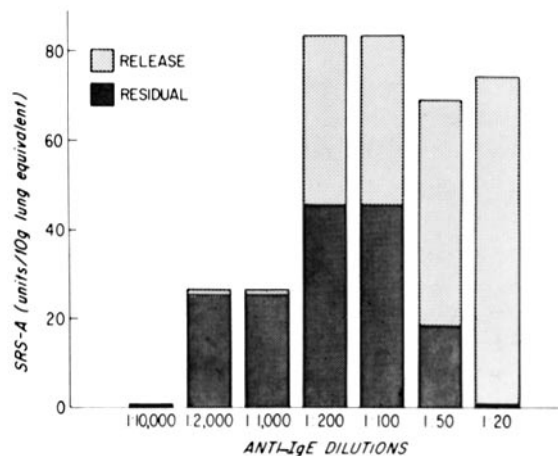


FIG. 3. SRS-A generation and release from isolated human lung cells challenged for 30 min by varying concentrations of anti-IgE.

ACTIVATION BY IgE-ANTIGEN INTERACTION. The time-course of total SRS-A generation in passively sensitized human lung fragments as determined by the sum of SRS-A accumulated in the tissue and that released into the diffusate after antigen challenge is depicted in Fig. 4. SRS-A was undetectable in the sensitized tissues at time zero, but accumulated rapidly after antigen challenge, reaching the maximum tissue level observed within 2 min. On the other hand, SRS-A

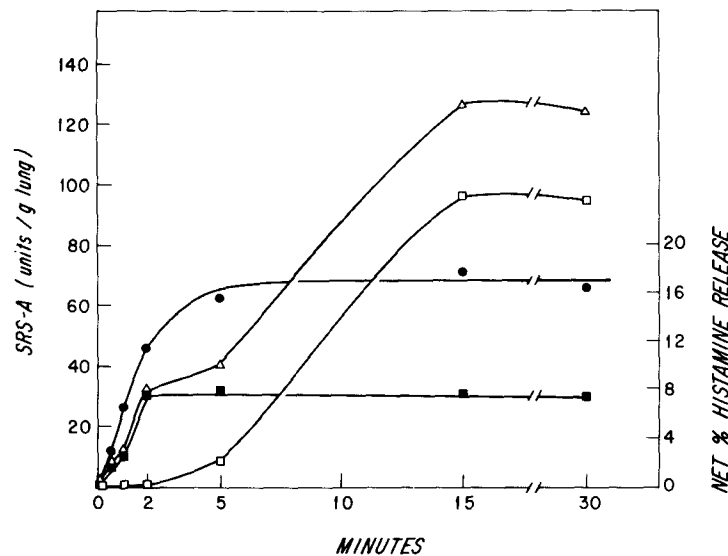


FIG. 4. Time-course of SRS-A generation and release from passively sensitized human lung fragments challenged with antigen E. ■, SRS-A residual; □, SRS-A release; Δ, SRS-A total; and ●, histamine release.

release into the diffusate was not evident until 2–5 min after antigen challenge and increased over 15 min, reflecting continued generation. Histamine release began within the 1st min and reached near maximum at 2 min, paralleling SRS-A generation before release.

Isolated passively sensitized lung cells challenged with specific antigen resembled the whole tissue in demonstrating a lag time between the accumulation of SRS-A in cells and its release (Fig. 5). SRS-A was undetectable in the cells at time zero, but appeared rapidly, reaching the maximum level observed by 1 min. Release into the supernate was not evident until 5 min and increased until 30 min, reflecting continued generation. Histamine release was maximal at 2 min, paralleling cellular SRS-A generation before release.

In order to examine the relationship between the magnitude of passive sensitization as assessed by antigen-induced histamine release and the generation of SRS-A, isolated lung cells were passively sensitized with varying doses of atopic serum (Fig. 6) and challenged with a fixed dose of antigen. While cells interacted with dilutions of 1:4 and 1:2 of atopic serum released histamine upon antigen challenge, those interacted with dilutions of 1:16 and 1:8 did not, implying that the lesser concentrations of IgE failed to sensitize the target cells. On the other hand, cells interacted with 1:16 and 1:8 dilutions of antiserum accumulated SRS-A after antigen challenge in the absence of SRS-A release. Unsensitized and antigen-exposed cells did not contain SRS-A. Thus, as with reversed anaphylactic challenge, the cellular accumulation of SRS-A without release reflects a minimal but definite immunologic activation. Sensitization at the greater concentrations of atopic serum followed by antigen challenge was not only accompanied by release of SRS-A into the supernate, but also by an incremental increase in total SRS-A generation.

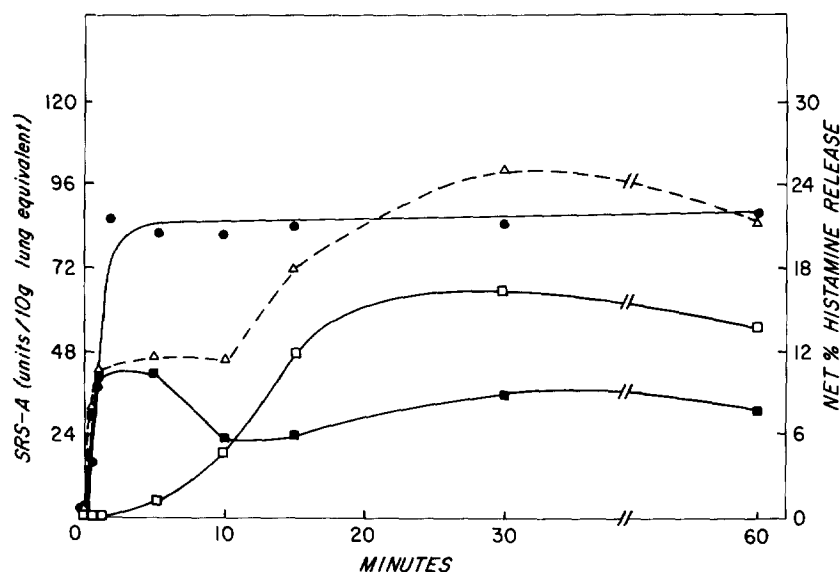


FIG. 5. Time-course of SRS-A generation and release from passively sensitized isolated human lung cells challenged with antigen E. ■, SRS-A residual; □, SRS-A release; △, SRS-A total; and ●, histamine.

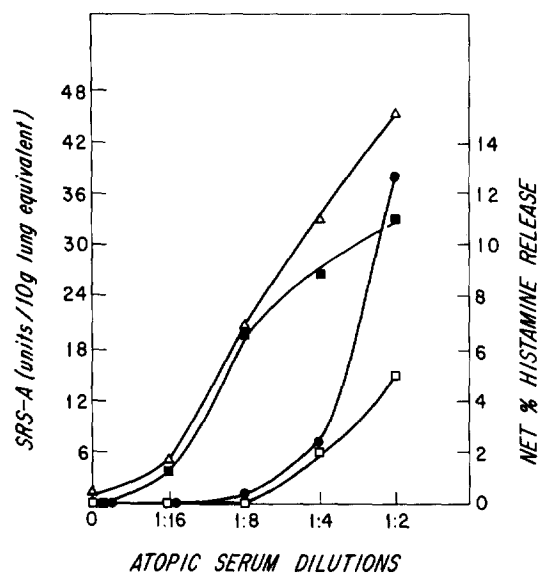


FIG. 6. SRS-A generation and release from isolated human lung cells passively sensitized with varying concentrations of atopic serum and challenged with specific antigen. ■, SRS-A residual; □, SRS-A release; △, SRS-A total; and ●, histamine release.

Partial Purification of Extracted and Released SRS-A. In order to establish that the material extracted and released and designated as SRS-A by bioassay on the atropinized antihistamine-treated guinea pig ileum did indeed represent SRS-A activity, partial purification (1) was carried out (Table I). In this

TABLE I
Partial Purification of SRS-A*

Procedure	Recovery of supernatant SRS-A	Recovery of residual SRS-A
	<i>U</i>	<i>U</i>
Starting material	100	240
Ethanol extraction	75	225
0.1 N NaOH (37°C × 30 min); Am- berlite XAD-2 chromatography	65	180
Silicic acid chromatography:		
Hexane	<5	<5
Methylene chloride	<5	<5
Acetone	<5	<5
n-Propanol	<5	<5
Ethanol:concentrated ammonia: water (6:3:1, vol/vol)	35	100

*Generated from a single 250-mg portion of passively sensitized human lung fragments 30 min after antigen challenge as described in the Materials and Methods.

procedure, cleavage of phospholipids is accomplished by base hydrolysis; histamine, serotonin, and the kinins are removed by their elution in the water wash from Amberlite XAD-2; and prostaglandins are extracted in hexane and acetone during silicic acid chromatography. SRS-A is stable to base hydrolysis, elutes in 80% ethanol on Amberlite XAD-2 chromatography, and remains on the silicic acid until the application of ethanol:concentrated ammonia:water (6:3:1). As shown in Table I, the recovery of SRS-A was 35 of 100 U in the initial supernate and 100 of 240 U in the initial extract of residual mediator. Although a 35-42% recovery is somewhat less than that observed when 1,000 times as much material was processed (1), it is completely characteristic of the recoveries repeatedly obtained with small-scale purification. A similar recovery was observed for supernatant and cellular SRS-A recovered from tissues or isolated cells challenged with anti-IgE.

Modulation of SRS-A Generation by Action of Cyclic Nucleotides. The capacity to measure total SRS-A generation as the sum of that in the tissues and that released into the supernate prompted an analysis of the effect of cyclic nucleotides upon the generation of SRS-A. It had previously been observed that the endogenous elevation of cyclic AMP or the addition of exogenous dibutyryl cyclic AMP inhibited SRS-A release (13, 29), while cholinergic stimulation or the introduction of 8-bromo-cyclic GMP enhanced release (19); but these experiments did not distinguish an action on generation separate from release. Human lung fragments that were passively sensitized revealed no detectable SRS-A after 2- or 30-min incubation in buffer, while antigen challenge resulted in generation of 30 U of SRS-A/g lung tissue at 2 min and 280 U/g at 30 min (Fig. 7). At 2 min, none of the SRS-A generated had been released into the fluid, while at 30 min, approximately 67% had been discharged. Preincubation of the fragments for 20 min with 10^{-3} M dibutyryl cyclic AMP completely prevented SRS-A generation

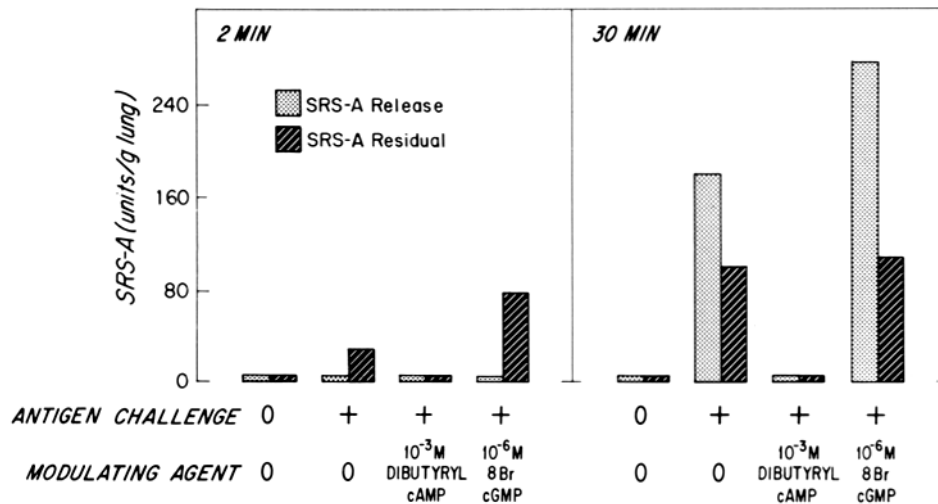


FIG. 7. Cyclic nucleotide modulation of SRS-A generation from passively sensitized human lung fragments.

at 2 and 30 min, in that no SRS-A was extractable from the tissue and none was found in the supernate. Preincubation of the fragments for 20 min with 10^{-6} M 8-bromo-cyclic GMP increased SRS-A generation at 2 min from 30 to 80 U and at 30 min from 280 to 390 U. Despite the 2.5-fold increase in SRS-A generation at 2 min, the product was entirely within the tissues, while at 30 min, 75% of that generated had been released.

Discussion

The capacity to extract SRS-A from human lung tissue or cells after immunologic activation and the measurement of SRS-A in both the extract and the surrounding fluid permit an analysis of SRS-A generation. That the SRS-A extracted was not merely trapped in interstitial spaces after release was indicated by its detection not only within tissue (Figs. 1 and 4) but within isolated washed cells after activation by reversed (Figs. 2 and 3) or direct (Figs. 5 and 6) anaphylaxis. The propensity of SRS-A to adsorb to a variety of surfaces as well as to certain plasma proteins (4, 30) does not account for its extraction from cells, since thorough washing did not diminish its association with the cell fraction and, more importantly, the total SRS-A generated did not bear a fixed relationship to the fraction remaining within the cells (Fig. 3). Further, under three circumstances the SRS-A detected was entirely intracellular, implying that release had not yet occurred. These conditions included: the initial 1–2 min of SRS-A generation after activation by reversed anaphylaxis (Fig. 1) in lung fragments and by direct anaphylaxis in passively sensitized lung fragments (Fig. 4) and isolated cells (Fig. 5); the response of isolated cells to limited immunologic activation as achieved by challenge with low doses of anti-IgE (Fig. 3) or passive sensitization with low concentrations of atopic serum before antigen challenge (Fig. 6); and the action of exogenous 8-bromo-cyclic GMP which yielded a

2.5-fold increase in the quantity of SRS-A generation without release at 2 min in passively sensitized antigen-challenged lung fragments (Fig. 7).

That the material extracted was indeed SRS-A was established by both differential bioassay and purification. Both the tissue and cell extract and supernatant SRS-A failed to contract the isolated gerbil colon (23) at a concentration five times that yielding a 1 U response on the isolated guinea pig ileum pretreated with atropine and antihistamine. During purification of the ethanol extract of tissues, cells, or diffusates, all contractile activity for the isolated atropinized antihistamine-treated guinea pig ileum eluted with ethanol from Amberlite XAD-2 and not with distilled water. Further, during silicic acid chromatography, no activity appeared with solvents capable of eluting prostaglandins, and recovery was limited to the solvent previously established as selectively eluting SRS-A (1).

The quantitation of SRS-A in tissues and cells as well as in the surrounding medium introduces an additional dimension to the study of SRS-A generation and may well clarify certain previously recognized differences between the elaboration of this mediator and those known to be preformed. The capacity of certain pharmacologic agents such as diisopropyl fluorophosphate (DFP) (31), isoproterenol in the presence of carbamylcholine (32), and the cytochalasins A and B (33) to suppress SRS-A release without affecting or even enhancing histamine release may well be explicable by an action or formation with or without an additional effect on release. Treatment of lung fragments with DFP (31) followed 3-5 min later by washing and antigen challenge permitted histamine release may well be explicable by an action or formation with or release if antigen challenge was carried out in the presence of DFP. Thus, the release of histamine requires the activation of an esterase from its DFP-resistant precursor form (34). In contrast, removal of DFP before antigen challenge did not relieve the inhibitory effect on SRS-A release (31, 34), implying that its generation involves an already active esterase. Stimulation of lung tissue with a fixed dose of isoproterenol sufficient to give a 10-fold increase in total tissue cyclic AMP completely prevented the subsequent appearance of histamine and SRS-A in the diffusate after antigen challenge. The treatment of replicate fragments with increasing doses of the cholinomimetic agent carbamylcholine progressively reversed the inhibition of histamine release and, at the highest dose, yielded enhancement. In contrast, there was little diminution of the isoproterenol-induced rise in total tissue cyclic AMP and no reversal of the total suppression of SRS-A release (32). In view of the suppression of SRS-A generation by cyclic AMP (Fig. 7), it may well be that the capacity of cholinergic stimulation to reverse adrenergic effects is more readily observed upon the secretory phase of mediator release than upon the generation of SRS-A. Similarly, the cytochalasins A and B, with an apparent site of action distinctly different from DFP or the cyclic nucleotides, suppressed the release of SRS-A at concentrations which fully permitted or even facilitated histamine release (33). As in each instance it is SRS-A release that fails to occur in association with histamine release, the observations could be unified by preferential action of the agents on SRS-A formation.

Another finding was that the intensity of the immunologic stimulus appeared to determine both the quantity of the SRS-A generated and its distribution between the cells and the fluid phase. Indeed, the presence of cellular SRS-A was a more sensitive index of immunologic activation than its detection in the surrounding fluid. Challenge of unsensitized cells with low concentrations of anti-IgE (Fig. 3) or sensitization with low concentrations of atopic serum followed by antigen challenge (Fig. 6) resulted in the accumulation of cellular SRS-A without release. Antigen-induced SRS-A formation in lung cells was observed without either histamine or SRS-A release (Fig. 6). Although the possibility of a preferential inactivation of mediators after release cannot be excluded, there is no evidence to support such an interpretation. As the intensity of the immunologic activation increased, there was not only a progressive shift of the detectable SRS-A to the surrounding medium, but also an increased generation (Figs. 3 and 6).

An additional consideration in the study of SRS-A generation relates to the possibility of more than one intracellular source or more than one cell source, either by primary immunologic activation or secondary to the action of a primary chemical mediator on a second cell. Such a possibility is introduced because of the continued generation of SRS-A at a time when the tissue (Figs. 1 and 4) or cellular (Figs. 2 and 5) content has plateaued and the release of preformed mediators is complete. The release of platelet-activating factor is an example of a primary mediator recruiting additional mediators through an effect on a second cell (35). The release of SRS-A from the rat peritoneal cavity by both IgE and mast cell-dependent (36) and IgG_a, complement, and polymorphonuclear leukocyte-dependent (37) mechanisms illustrates that more than one cell type can participate in its generation. Another alternative is that the IgE immunoglobulins bound to mast cells or basophils in excess of those required for optimal release of preformed mediators interact with antigen to perturbate the membrane with resultant SRS-A generation independent of intracellular accumulation or of the discharge of preformed mediators.

Summary

The capacity to extract slow-reacting substance of anaphylaxis (SRS-A) from human lung tissue or cells after immunologic activation, together with the measurement of SRS-A in both the extract and the surrounding fluid, permits study of total SRS-A generation. That the material extracted is SRS-A was established by both differential bioassay and purification. SRS-A accumulation was entirely intracellular after limited IgE-dependent direct or reversed anaphylactic activation. Intracellular accumulation also generally preceded release, with generation of SRS-A continuing well beyond a plateau in the cellular SRS-A level and the release of preformed mediators. The quantity of SRS-A generated after immunologic activation was modulated by the introduction of exogenous cyclic nucleotides, revealing a site of cyclic nucleotide action distinct from that on mediator release. The capacity to determine not only the release of preformed mediators but also the generation of a newly formed mediator, the

sum of SRS-A in cells and supernate, adds an additional dimension to the analysis of the cellular events of immediate hypersensitivity.

The excellent technical assistance of Ms. Janet Woods is gratefully acknowledged. We also acknowledge the assistance of the surgical and operating room staffs of the New England Baptist Hospital, Boston, Mass., the New England Deaconess Hospital, Boston, Mass., and the Overholt Clinic, Boston, Mass., in providing lung specimens.

Received for publication 8 July 1974.

Bibliography

1. Orange, R. P., R. C. Murphy, M. L. Karnovsky, and K. F. Austen. 1973. The physicochemical characteristics and purification of slow-reacting substance of anaphylaxis. *J. Immunol.* **110**:760.
2. Orange, R. P., R. C. Murphy, and K. F. Austen. 1974. Inactivation of slow reacting substance of anaphylaxis (SRS-A) by arylsulphatases. *J. Immunol.* **113**:316.
3. Orange, R. P., D. J. Stechschulte, and K. F. Austen. 1969. Cellular mechanisms involved in the release of slow reacting substance of anaphylaxis. *Fed. Proc.* **28**:1710.
4. Brocklehurst, W. E. 1962. Slow reacting substance and related compounds. Progress in Allergy, Vol. 6. Karger AG., S., Basel, Switzerland/New York. 539.
5. Kellaway, D. H., and E. R. Trethewie. 1940. The liberation of a slow-reacting smooth-muscle stimulating substance in anaphylaxis. *Q. J. Exp. Physiol.* **30**:121.
6. Brocklehurst, W. E. 1953. Occurrence of an unidentified substance during anaphylactic shock in cavy lung. *J. Physiol. (Lond.)* **120**:16P.
7. Brocklehurst, W. E. 1960. The release of histamine and formation of slow-reacting substance (SRS-A) during anaphylactic shock. *J. Physiol. (Lond.)* **151**:416.
8. Orange, R. P., M. D. Valentine, and K. F. Austen. 1968. Antigen-induced release of slow reacting substance of anaphylaxis (SRS-A^{rat}) in rats prepared with homologous antibody. *J. Exp. Med.* **127**:767.
9. Stechschulte, D. J., R. P. Orange, and K. F. Austen. 1973. Detection of slow reacting substance of anaphylaxis (SRS-A) in plasma of guinea pigs during anaphylaxis. *J. Immunol.* **111**:1585.
10. Stechschulte, D. J., K. F. Austen, and K. J. Bloch. 1967. Antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A) in the guinea pig and rat. *J. Exp. Med.* **125**:127.
11. Ishizaka, T., K. Ishizaka, R. P. Orange, and K. F. Austen. 1971. Pharmacological inhibition of the antigen-induced release of histamine and slow reacting substance of anaphylaxis (SRS-A) from monkey lung tissues mediated by human IgE. *J. Immunol.* **106**:1267.
12. Sheard, P., P. G. Killingback, and A. M. J. N. Blair. 1967. Antigen-induced release of histamine and SRS-A from human lung passively sensitized with reaginic serum. *Nature (Lond.)* **216**:283.
13. Orange, R. P., W. G. Austen, and K. F. Austen. 1971. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. I. Modulation by agents influencing cellular levels of cyclic 3',5'-adenosine monophosphate. *J. Exp. Med.* **134**:136S.
14. Ishizaka, T., K. Ishizaka, and H. Tomioka. 1972. Release of histamine and slow reacting substance of anaphylaxis (SRS-A) by IgE-anti-IgE reactions on monkey mast cells. *J. Immunol.* **108**:513.

15. Grant, J. A., and L. M. Lichtenstein. 1974. Release of slow reacting substance of anaphylaxis from human leukocytes. *J. Immunol.* **112**:897.
16. Parish, W. E. 1973. Reaginic and nonreaginic antibody reactions on anaphylactic participating cells. In *Mechanisms in Allergy*. L. Goodfriend, A. H. Sehon, and R. P. Orange, editors. Marcel Dekker, Inc., New York. 197.
17. Kaliner, M., S. I. Wasserman, and K. F. Austen. 1973. The immunologic release of chemical mediators from human nasal polyps. *N. Engl. J. Med.* **289**:277.
18. Böyum, A. 1968. Isolation of leucocytes from human blood, further observations. *Scand. J. Clin. Lab. Invest. Suppl.* **97**:21:31.
19. Kaliner, M., R. P. Orange, and K. F. Austen. 1972. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. IV. Enhancement by cholinergic and alpha adrenergic stimulation. *J. Exp. Med.* **136**:556.
20. Ogawa, M., S. Kochwa, C. Smith, K. Ishizaka, and D. R. McIntyre. 1969. Clinical aspects of IgE myeloma. *N. Engl. J. Med.* **281**:1217.
21. Axen, R., J. Porath, and S. Ernback. 1967. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature (Lond.)*. **214**:1302.
22. Tyrode, M. V. 1910. The mode of action of some purgative salts. *Arch. Int. Pharmacodyn. Ther.* **20**:205.
23. Orange, R. P., and K. F. Austen. 1974. The biological assay of slow reacting substances. In *Methods in Immunology and Immunochemistry*, Vol. 5. M. Chase and C. A. Williams, editors. Academic Press Inc., New York. In press.
24. Kay, A. B. 1970. Studies on eosinophil leukocyte migration. II. Factors specifically chemotactic for eosinophils and neutrophils generated from guinea pig serum by antigen-antibody complexes. *Clin. Exp. Immunol.* **7**:723.
25. Boyden, S. 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. *J. Exp. Med.* **115**:453.
26. Day, R. P. 1970. Eosinophil cell separation from human peripheral blood. *Immunology*. **18**:955.
27. Gould, K. G., J. A. Clements, A. L. Jones, and F. M. Felts. 1972. Dispersal of rabbit lung into individual viable cells: a new method for the study of lung metabolism. *Science (Wash. D. C.)*. **178**:1209.
28. Wasserman, S. I., E. J. Goetzel, and K. F. Austen. 1974. Preformed eosinophil chemotactic factor of anaphylaxis (ECF-A). *J. Immunol.* **112**:351.
29. Orange, R. P., M. A. Kaliner, P. J. LaRaia, and K. F. Austen. 1971. The immunological release of histamine and slow reacting substance of anaphylaxis from human lung. II. Influence of cellular levels of cyclic AMP. *Fed. Proc.* **30**:1725.
30. Middleton, E., and G. B. Phillips. 1964. Distribution and properties of anaphylactic and venom-induced slow reacting substance and histamine in guinea pigs. *J. Immunol.* **93**:220.
31. Orange, R. P., M. A. Kaliner, and K. F. Austen. 1971. The immunological release of histamine and slow reacting substance of anaphylaxis from human lung. III. Biochemical control mechanisms involved in the immunologic release of the chemical mediators. In *Biochemistry of the Acute Allergic Reactions*, Second International Symposium. K. F. Austen and E. L. Becker, editors. Blackwell Scientific Publications Ltd., Oxford, England. 189.
32. Kaliner, M. A., and K. F. Austen. 1974. The hormonal control of the immunologic release of histamine and slow reacting substance of anaphylaxis from human lung. In *Cyclic AMP, Cell Growth, and the Immune Response*. W. Braun, L. M. Lichtenstein and C. W. Parker, editors. Springer-Verlag New York Inc., New York. 163.
33. Orange, R. P. 1974. Dissociation of the *in vitro* immunologic release of histamine and

- slow reacting substance of anaphylaxis (SRS-A) from human lung using cytochalasins A and B. *Fed. Proc.* **33**:761. (Abstr.)
34. Kaliner, M. A., and K. F. Austen. 1973. A sequence of biochemical events in the antigen-induced release of chemical mediators from sensitized human lung tissue. *J. Exp. Med.* **138**:1077.
 35. Benveniste, J., P. M. Henson, and C. G. Cochrane. 1972. Leukocyte-dependent histamine release from rabbit platelets. *J. Exp. Med.* **136**:1356.
 36. Orange, R. P., D. J. Stechschulte, and K. F. Austen. 1970. Immunochemical and biologic properties of rat IgE. II. Capacity to mediate the immunologic release of histamine and slow reacting substance of anaphylaxis (SRS-A). *J. Immunol.* **105**:1087.
 37. Morse, H. C., III, K. J. Bloch, and K. F. Austen. 1968. Biologic properties of rat antibodies. II. Time-course of appearance of antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A^{rat}); association of this activity with rat IgG_a. *J. Immunol.* **101**:658.